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BLOOD-CULTURE STUDIES ON TYPHUS EXANTHEMATICUS IN SERBIA, BULGARIA, AND RUSSIA *

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The blood-culture studies which form the basis of the present report were made by us in the Balkans and in Volhynia (Russia) and Galicia during the spring and Winter of 1915-1916. They represent part of the work of an expedition which in June, 1915, left America for Serbia under the auspices of the American Red Cross, for the purpose of carrying on investigations concerning the cause and prevention of typhus fever. For all the expenses of the expedition, including the purchase of a complete laboratory equipment, we are indebted to the Board of Directors of the Mount Sinai Hospital.

The investigations were actually begun in Serbia only after the occupation of the country by the Bulgarians (October 1915) and were subsequently assisted in every possible manner by the Bulgarian and German military authorities. In January, 1916, the work was also started in Volhynia (Russia) by one of us (Baehr) at the invitation of the Austro-Hungarian Government. During the next few months we were therefore able to carry on the investigations independently in different parts of Europe.

The present communication is limited to the results of blood-culture studies. Reports concerning the serologic and epidemiologic investigations and one dealing with the results of the studies on prophylactic immunization against typhus fever will appear in subsequent numbers of this journal.

The cultivation by anaerobic methods, of an organism from the blood of individuals suffering from typhus fever was first reported in 1914 by Plotz.[1] In 1915 an extensive report dealing with the evidence that this organism is the causative factor in typhus fever was published by Plotz, Olitsky, and Baehr.[2] They reported the isolation of the bacterium from the blood in pure culture in 18 of 34

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* Received for publication November 27, 1916. This work was done during the tenure of the Theodor Escherich and George Blumenthal Jr. fellowships in pathology.

[1] Jour. Am. Med. Assn., 1914, 62, p. 1556.

[2] Jour. Infect. Dis., 1915, 17, p. 1.

cases of the mild type of the disease endemic among the Russian immigrants in New York (so-called Brill's disease[3]), or 53% of the cases studied, and in 7 cases of the severe European epidemic typhus, or 100% of the cases of this type studied bacteriologically during the febrile period of the disease. Similar blood cultures made on 198 control cases yielded no such organism. In the work which forms the subject of this report, bacteriologic, serologic, and experimental observations were reported which confirmed the opinion as to the etiologic significance of this bacterium in typhus exanthematicus.

Confirmation of these observations by American bacteriologists has thus far been limited by the rarity of typhus fever in this country. For, with the exception of Mexico, typhus fever in America at the present time is restricted almost exclusively to the sporadic cases occurring among the Russian immigrant population of New York. Bernstein[4] and Blatteis and Lederer[5] recovered the organism from such cases of typhus fever. During our absence from the country, Gottesman and Klein isolated the organism from over 50% of the cases admitted to the Mount Sinai Hospital. Anderson[6] succeeded in cultivating B. typhi-exanthematici from the blood of guinea-pigs which had been infected with the blood of individuals suffering from typhus fever.

During the Serbian epidemic of 1914-15 Zinsser and Hopkins[7] were able to recover the organism from only 3 of a large series of typhus cases. But, as explained by Zinsser, the proper ascitic fluid for most of his cultures was not available and the technic necessary for the successful cultivation of the organism was therefore often impossible. More recently, however, Olitsky, Denzer, and Husk,[8] working in Mexico, reported the successful isolation of B. typhi-exanthematici from 8 cases of the severe form of the disease at present epidemic in that country, or 100% of the cases studied bacteriologically.

In Europe, confirmatory reports were published from two different sources. The cultivation of B. typhi-exanthematici in Serbia from the blood of individuals suffering from typhus fever was reported by

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[3] New York Med. Jour., 1898, 67, pp. 48, 77. Am. Jour. Med. Sc., 1910, 139, p. 484; 1911, 162, p. 196.

[4] Personal communication.

[5] Long Island Med. Jour., 1916, 10, p. 169.

[6] Quoted by Zinsser, Jour. Lab. and Clin. Med., 1916, 1, p. 623.

[7] Jour. Lab. and Clin. Med., 1916, 1, p. 623. Hiss and Zinsser, Textbook of Bacteriology, 1916.

[8] Jour. Infect. Dis., 1916, 19, p. 811.

Popoff,[9] and in Volhynia (Russia) by Paneth.[10] Mühlens and Ficker,[11] working in Serbia, also were able to recover the organism from the blood of typhus patients and not from control cases, and Ghon in Prague isolated it from the blood of guinea-pigs which had been infected with the blood of typhus patients. Up to the time of our departure from Europe, June 1916, the reports of the last two observers had not yet appeared in print. Popoff isolated the organism in pure culture from the blood of 6 of 10 cases, Paneth in 2 of 5, Mühlens and Ficker in 12 of 20 cases.

BLOOD-CULTURE METHOD

The method followed in making the anaerobic blood culture was the Liborius-Veillon,[12] using the ascitic-fluid glucose agar recommended by Libman as the optimal medium for bacterial cultivation.[13] The technic employed was briefly described in our previous communication. In view of the fact that some observers have failed to understand the technic, the procedure will be redescribed so as to include some details which were previously omitted.

The Ascitic or Hydrocele Fluid.—The essential ingredient in the medium is ascitic or hydrocele fluid. It must be clear, free of bile or blood pigment, and possessed of a specific gravity of more than 1.015. A filtered ascitic fluid or one which contains a preservative or which has been sterilized by heating should not be used. Pleural fluids are also valueless.

Before use, the ascitic or hydrocele fluid should be tested both aerobically and anaerobically as to its sterility. In view of the possible presence of hemoglobinophilic anaerobes as contaminations in ascitic fluid, it is always advisable in carrying out the anaerobic test to use the ascitic fluid in blood cultures on nontyphus controls. Even if all the tubes remain apparently sterile, these anaerobic control cultures should be observed in a thermostat for not less than 21 days.

Preparation of Medium.—The agar employed was prepared according to modifications described by Canary.[14] The following ingredients are placed in a 4- or 5-liter flask:

Distilled water	3000	c.c.
Liebig's meat extract	12	gm.
Sodium chlorid	15	gm.
Peptone (Witte)	30	gm.
Normal sulfuric acid	17.5	c.c.
Agar shreds	85	gm.

The flask is placed in an Arnold steam sterilizer for 2 hours, after which the contents are rapidly filtered into a pot through several layers of gauze to remove the coarser impurities. Meanwhile the whites of 8 eggs are thoroughly mixed in 150 c.c. of distilled water. The fluid agar is then gradually cooled to 55 C., with constant stirring, and returned to the large flask, which has meanwhile been cleaned. To this the egg mixture is added. The flask is now thoroughly shaken in order to mix the contents and then is placed again in the Arnold steam sterilizer for 2 hours. It is important that the Arnold sterilizer be under full steam—that is, that the interior be at least 100 C. before the flask is introduced. On removing the flask from the Arnold, the egg white should be coagulated into a firm clump and the agar absolutely clear. This clear agar is carefully decanted and filtered through moistened absorbent cotton.

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[9] Deutsch. med. Wchnschr., 1916.

[10] Med Klin., 1916.

[11] Personal communication.

[12] Quoted by Besson, Technique microbiologique et serotherapique, 1914, p. 105.

[13] Jour. Med. Research, 1901, 1, p. 84. Bull. Johns Hopkins Hosp., 1906, 17, p. 215. Libman, Celler, and Sophian, paper read before Association of American Physicians, 1914 (not published yet).

[14] Tr. New York Pathol. Soc., 1905, 5, p. 132.

The agar is then titrated in the following manner. About 100 c.c. distilled water is brought to boil in a porcelain dish. It is then removed from the flame, and 5 c.c. of the fluid agar and then a few drops of a 0.5% alcoholic solution of phenolphthalein are added. The hot agar being constantly stirred, a 1:40 normal sodium-hydrate solution is slowly dropped into it until the first faintest evidence of pink appears throughout the mixture. This is the end point. If the ingredients of the agar have previously been properly measured out, the end point in the titration should be reached when 1.8-2.2 c.c. of the 1:40 normal sodium-hydrate solution have been added.

Let us assume that 2 c.c. happens to be the amount needed to neutralize the 5 c.c. of agar. Then in order to neutralize 100 c.c. of agar, 40 c.c. of a 1:40 normal sodium-hydrate solution would be needed. This is equivalent to 1 c.c. of a normal sodium-hydrate solution, so that the acidity of the agar in question may be called 1.

In our blood cultures, agar was never used which had an acidity of less than 0.9 or more than 1.1. For each 0.1 variation, 1 c.c. of a normal sulfuric-acid solution is added to each liter of agar if too alkaline, or 1 c.c. of a normal sodium-hydrate solution if too acid. The agar is then retitrated. It is seldom, however, that the acidity falls outside the proper limits of 0.9-1.1.

After the titration is completed, 2% glucose is added to the fluid agar. Test tubes measuring 20 cm. in length and 2 cm. in diameter are then filled about half full with the medium (about 20 c.c. in each tube) and sterilized once in an autoclave at 10 kg. pressure for 20 minutes or in an Arnold sterilizer for 20 minutes on 3 or 4 successive days.

Technic of Blood Culture.—In making the blood culture, the 2% glucose agar in 8 of these test tubes is reduced to a fluid by boiling and then cooled to 40-42 C. The skin over the antecubital space on the patient's arm is thoroughly disinfected, preferably by alcohol followed by tincture of iodin. A sterile 15-c.c. syringe and needle are used for obtaining the blood from the median basilic or median cephalic veins. The syringe should not be used until it has cooled.

Having obtained a syringe full of blood, it is now important to work very rapidly, otherwise the blood will coagulate or the medium will solidify. The needle is removed from the syringe by means of a forceps, the tip of which has been passed several times through a flame, and the tip of the syringe also flamed off twice very rapidly. The 15 c.c. of blood are then divided among the 8 tubes of glucose agar, each tube receiving approximately 2 c.c. To each tube in turn, ascitic fluid equivalent to at least one third of the volume of the agar (i. e., 6-10 c.c.) is added. The contents, consisting of glucose agar, blood, and ascitic fluid are now poured once or twice into another sterile test tube so as to secure thorough mixing. It is important, in carrying out the mixing, that the introduction of air bubbles into the medium be avoided. After thorough solidification of the mixture, each tube is then covered with a layer of plain agar, 2 or 3 cm. deep. Finally, in order to prevent drying-out of the medium, it is often advisable to paraffin the cotton stopper of each tube.

Examination of Colonies.—After being placed in a thermostat at 37 C., colonies usually appear in from 7 to 14 days, tho in a few instances they have been observed as early as the 5th day. Colonies may also appear any time up to the 19th day. Tubes which appear to be negative should therefore be observed for at least 3 weeks and should then be opened and carefully examined.

In order to open a tube a circular scratch is made on the glass near the lower part of the tube and the glass is cracked at this point by a slight blow. By pushing the cotton stopper into the tube, the column of agar can be made to slide into a sterile petri dish. If a colony is seen, the surface over it is seared with a hot knife or a hot platinum loop. The agar over the colony is then dug away with the loop until the colony is reached and a small portion of it is used for a Gram stain. The remainder of the colony is subinoculated on one or more slants of 0.5% glucose ascitic-fluid agar, and grown in a Buchner tube half filled with equal parts of pyrogallic acid and 30% sodium hydrate. The rest of the original blood-agar column is finally cut into thin slices with a hot knife, so that colonies in the interior will not be overlooked.

In making subinoculations it is important that much material (bacteria) be used and that it be thickly smeared on the surface of the slants. This is essential for successful growth, especially in the first few subinoculations.

The slants used by us for subinoculations consisted of agar prepared in the prescribed manner containing 0.5% or 2% glucose and one third by volume of good ascitic fluid. Subcultures were reinoculated every 7-10 days.

MORPHOLOGIC IDENTIFICATION OF THE ORGANISM

The organism recovered by us in the Balkans and in Russia from the blood of individuals with typhus fever was identical in all respects with the one isolated by us from typhus patients in New York, in Russia by Paneth,[10] in Serbia by Popoff,[9] and in Mexico by Olitsky, Denzer, and Husk.[8]

Colonies.—In our Russian series of blood cultures, the average time which elapsed before colonies first appeared in the culture tubes was between 8 and 9 days. The time varied, however, in the different blood cultures from 5 to 15 days. In our Balkan series, the average time which elapsed between the time when the blood cultures were taken and the time when the colonies became visible was somewhat longer, 10-11 days, the limits being 6-17 days. This greater interval in the Balkan studies is undoubtedly due to the fact that we were unable to observe the cultures daily, so that colonies were often not noted until some days after they had probably reached a visible size. The average time of 8-9 days observed in the Russian studies, in which daily observations of the culture tubes were made, corresponds almost exactly with the results obtained by us in our American work and by Olitsky, Denzer, and Husk in their Mexican studies.

In 4 blood cultures of the Russian series (Cultures 6, 15, 18, and 25) colonies developed only in the interior of the tubes, and were therefore not visible through the opaque medium. They were discovered therefore only when the tubes were broken open and the medium cut into slices, that is, 24, 25, 26, and 29 days, respectively, after the cultures were made (see Table 3). These four blood cultures have not been included in the average mentioned, for it is impossible to say when the colonies first appeared. In 3 blood cultures we have observed the development of a colony in the center of a blood clot. This was also observed in the New York studies.

The colony first appears as a small opaque spot which in direct light is white in color. During the subsequent 2-3 days it grows rapidly larger, usually assuming a Y shape, and develops a brownish zone of precipitation in the medium immediately about it. The maximal size (5 mm. in its longest diameter) is reached within a few days after the colony first becomes visible; by this time the precipitation zone around it is also well developed. On cross-section, the colony is usually Y-shaped, each limb of the Y being flattened into one plane. In consistency the growth is always exceedingly soft, and the color, especially of the older colonies, is pale brown.

One of the unusual features in the development of the colony is its initial slow rate of growth—the colonies seldom reaching a visible size before the 8th or 9th day — followed by a second period of very rapid growth. In view of the long period before the colony becomes visible, its very rapid growth during the succeeding 2-3 days is rather striking. Apparently the organism must first adapt itself to the artificial medium, but once this adaptation has taken place, growth seems to proceed very rapidly.

It is not unlikely that some of the organisms present in the blood at the time of the culture do not succeed in adapting themselves to the medium and therefore never develop into colonies. To this we shall again have occasion to refer.

Morphology.—In size and general appearance the organism when stained with a simple anilin dye somewhat resembles the influenza bacillus. It is very small and slender, the average length being about a micron. In young cultures the organism is small and uniform in size. In very old subcultures there is often a mixture of variously sized bacilli, from coccoid forms up to some measuring 2 microns in length. The bacilli are usually straight, tho slightly curved forms occasionally occur. The ends are rounded or slightly pointed, and often show a tendency to fade off. With special stains (pyoktanin acetic-acid Bismarck brown) an occasional organism will show a fine polar body at one end, more rarely at both. There is no definite arrangement of the bacilli; some lie end to end, but just as many lie side by side or at an angle to one another.

Reaction to the Gram Stain.—The organism is gram-positive. In some of our blood cultures, however, smears made directly from the colonies showed only bacilli which were completely decolorized by Gram's method. In other blood cultures, the predominating organism in the colonies was gram-positive with numerous gram-negative bacilli scattered through the field. In subsequent subcultures, the bacilli always became gram-positive.

This observation has been made independently by Olitsky, Denzer, and Husk.[8] Like them, we have also made the observation that the bacilli seen in typhus-infected lice are decolorized by Gram's method. Da Rocha-Lima[15] in his recent studies found that these gram-negative bacilli are present in enormous number in the stomach wall of typhus-infected lice. Olitsky, Denzer, and Husk succeeded in cultivating the organism from such lice with the anaerobic methods here described, and observed the gram-negative forms gradually become gram-positive in subsequent transplants.

The gram-negative forms of B. typhi-exanthematici sometimes seen in the blood-culture colonies, as well as those seen in the stomach of the typhus-infected louse, are exceedingly minute in size, averaging only 0.5 micron in length. In view of our recent blood-culture observations there is a possibility that, just as in the infected louse, the organism may exist in the human body during the disease in this minute gram-negative form.

The gram-negative forms seen occasionally in early cultures from typhus patients and in typhus-infected lice stain distinctly with acid fuchsin and ordinary anilin dyes. They are not to be confounded with the gram-negative forms seen in smears from very old subcultures, which stain very palely and vaguely with fuchsin, and merely represent dead or degenerated bacilli.

Cultural Characteristics.—Because of the limitation of the facilities under which we were forced to work, it was not possible to transplant each strain of B. typhi-exanthematici isolated, on all the varieties of media used in our previous studies. The following cultural characteristics, together with the morphologic and serologic observations, were sufficient to determine the identity of the bacilli isolated by us from typhus patients in the Balkans and in Russia with those previously isolated in America.

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[15] Arch. f. Schiffs-u. Tropenhyg., 1916, 20, p. 17.

The organism is an obligate anaerobe. Six of the 43 strains isolated by us we finally succeeded in growing under aerobic conditions, but only after they had been kept on artificial media for 8 or 9 months. These 6 strains are still facultative anaerobes, growing much better under anaerobic conditions than under aerobic. When kept continuously for several generations under aerobic conditions all 6 showed a tendency to die out, only one strain surviving 4 successive transplantations.

The organism grows only on a medium containing ascitic fluid and glucose. Altho all strains have been cultivated artificially for many months, it has not yet been possible to obtain growth on solid medium which does not contain both ascitic fluid and glucose. The optimal medium contains 1 part ascitic fluid to 2 parts of 2% glucose agar. Even on the slants of this medium a very large amount of culture material (at least 1 loopful) must be used in order to obtain growth and it is even advisable to smear the culture material in thick streaks on the surface. It is quite possible that the bacilli in the superficial portion of the thick streaks through their reductase action improve the anaerobic conditions and in this way favor the growth of the organisms in the deeper portions of the streak.

Under the conditions mentioned the subcultures on slants of ascitic-fluid glucose agar incubated in Buchner tubes containing equal parts of pyrogallic acid and 30% sodium-hydrate solution usually show evidences of growth after about 3 days. The growth is profuse by the 6th or 7th day, and at that time is soft and creamy in character, raised from the surface and of a glistening white color. Very old subcultures may develop a pale-brownish or pale-reddish-brown tint. In the medium itself a diffuse clouding gradually develops as the growth increases — the precipitation phenomenon of Libman.[13]

In stab cultures on the same medium, growth develops in 4 or 5 days if the column of media be layered with petrolatum liquidum or agar. In these cultures the precipitation of the medium can be seen to spread outward from the site of the stab. We have found that if the stab cultures be properly layered and the cotton stoppers paraffined to prevent drying of the medium, the bacilli will often remain viable for as long as 3 months, even when kept at room temperature.

In a 2% glucose broth exceedingly slight growth may occur under anaerobic conditions in the form of minute flocculi which settle to the bottom of the tube. In glucose broth containing one third by volume of ascitic fluid the anaerobic growth is more marked, but after a few days the proteins of the ascitic fluid begin to come down. In fact, by the 5th or 6th day there is a heavy sediment in the bottom of the broth, which consists almost entirely of precipitated proteins with relatively few bacilli. As Libman[13] has already shown for some other organisms, this precipitate is chiefly due to the acidification of the medium resulting from the splitting up of glucose by the bacteria. By the 7th or 8th day the precipitate in the ascitic glucose broth has reached its maximum and the bacilli have almost ceased to multiply. By this time the medium, which before inoculation had an acidity of about 0.6 will, when titrated against phenolphthalein, be found to have reached an acidity of 4 or even 5. That is to say, 5 c.c. of a normal sodium-hydrate solution are needed to neutralize 100 c.c. of the broth.

In the previous work it was reported that the organisms fermented not only glucose with acid-production and precipitation, but also maltose, galactose, and inulin. Raffinose, arabinose, saccharose, lactose, dextrin, and mannite were not fermented. For the purpose of identification we inoculated our strains only on ascitic-fluid agar containing Kahlbaum's litmus and either glucose, lactose, or inulin. The results were identical with those obtained with the American and Mexican strains, glucose and inulin being fermented, but not lactose.* Growth was also always profuse on media containing glucose or inulin, but very scanty on a medium containing only lactose.

SEROLOGIC IDENTIFICATION OF THE ORGANISM

Agglutination with Immune Rabbit Serum.—For further purposes of identification agglutination tests were made with some of our strains, immune rabbit serum being used. Altho all the strains so tested were firmly agglutinated up to the maximal titer of the serum, we do not now consider these results of much importance. For it has recently been demonstrated by Olitsky and Bernstein[16] that the immune rabbit serum prepared artificially by the intravenous injection of organisms which have been grown on serum media may give nonspecific results.

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* For a method of purifying lactose for bacteriologic use, see Olitsky, Denzer, and Husk.[8]

[16] Jour. Infect. Dis., 1916, 19, p. 213.

This is due to the fact that such an immune serum contains, beside the specific antibody, antibodies developed against the serum (or protein) employed in the medium on which the bacteria have been grown. These serum antibodies give rise to nonspecific agglutinins, precipitins, complement-fixing bodies, and cellular antibodies. Such immune rabbit serum will, for example, agglutinate or precipitate not only the organism against which the animal has been immunized, but also totally different organisms which have merely been grown on the same ascitic-fluid medium.

Agglutination with Serum of Typhus Convalescents.—All the 41 strains isolated by us were agglutinated in high dilutions (the maximum being 1:1600) by the serum of typhus convalescents. For reasons enumerated in a previous paper the microscopic method was used. At times a given convalescent's serum would agglutinate some strains in one or more dilutions higher than other strains. In fact, the relative agglutinability of the different strains varied somewhat with the different sera. The difference was usually insignificant except in the case of strains which had only recently been isolated. These were sometimes inagglutinable by convalescent sera until they had been subinoculated a few times. On the other hand it was often possible to obtain agglutination in high dilutions with the organism obtained directly from colonies in the original blood cultures.

The details of the agglutination studies and their diagnostic significance will be reserved for one of the subsequent papers.

Complement-Fixation Tests.—In these tests (performed by Dr. Peter K. Olitsky) antigens prepared from the strains isolated in the Balkans, as well as from those isolated in Russia, gave strong complement-fixation with convalescent serum. As in the previous work, the antigen was prepared as follows: The growth on agar slants was suspended in distilled water, heated to 60 C. for 1 hour, then autolyzed at 37 C. for 24 hours and finally filtered through a Berkefeld candle (size N — pressure 100 mm. Hg.). The clear filtrate was used as antigen. Bacterial antigens which have not been passed through a Berkefeld filter are often anticomplementary and therefore not serviceable. Never more than one fourth of the anticomplementary unit was employed.

The hemolytic system consisted of antisheep rabbit amboceptor, 5% suspension of sheep cells, and guinea-pig serum (1:10) as complement. The quantities employed were one half of the amounts employed in the original Wassermann test. The serum used was inactivated at 56 C. for one-half hour and was absolutely nonanticomplementary.

The following table shows the identical results obtained with antigens made from New York, Balkan, and Russian strains (Table 1).

TABLE 1

COMPLEMENT-FIXATION WITH SERUM FROM A NEW YORK CASE OF TYPHUS FEVER

Amount of Serum from Typhus Convalescent (5th day) (c.c.)	Without Antigen	With Antigen		
		New York	Balkan	Russian
0.00	0	0	0	0
0.05	0	++++	++++	++++
0.10	0	++++	++++	++++

0 = no fixation. ++++ = complete fixation.

Table 1 also illustrates the cross-fixation obtained by using antigen prepared from Balkan and Russian strains of B. typhi-exanthematici, and serum from a New York case of typhus fever.

GENERAL RESULTS OF THE EUROPEAN STUDIES

In considering the results of the studies, it must be remembered that the work was carried out under exceedingly primitive conditions. In the Balkan series blood cultures were often made on patients several days' journey from our laboratory, and in some instances it was not possible to place the cultures in an incubator until 4 or 5 days after they had been taken. This, in our opinion, was responsible for negative results with certain cultures, which we have nevertheless included in this series, and which have undoubtedly served to lower the actual percentage of positive results (Table 2).

In the Russian series, the blood cultures with a few exceptions were placed in an incubator within 2 or 3 hours after they were taken. Only in one instance, Culture 7, did 24 hours intervene between the time when a blood culture was taken and the time when it was placed in an incubator. Culture 7 was positive.

The ascitic or hydrocele fluid used in blood cultures of both Balkan and Russian series answered all the requisites previously enumerated. In all, 11 ascitic fluids and 3 hydrocele fluids were employed, and the specific gravity of each was more than 1.020. Each fluid was tested anaerobically by taking a blood culture on a control case. The control cultures included typhus convalescents and patients suffering from various febrile conditions, such as relapsing fever, pneumonia, typhoid fever, and erysipelas. The controls were only made in order to determine the sterility of the ascitic fluid used, for our supplies were limited and we had already reported uniformly negative results in 198 individuals suffering from febrile conditions other than typhus fever.

Results of Balkan Studies. — Forty blood cultures were made in Serbia and Bulgaria on that number of persons suffering from typhus fever (Table 2). In 19 B. typhi-exanthematici was recovered, or in 47.5% of the cultures taken. For reasons just stated, this percentage is probably lower than it should have been. When, as in the Russian studies, it was possible to exercise the proper care in the technic, the percentage of positive results was much higher (Table 3).

TABLE 2

RESULTS OF BLOOD CULTURES IN TYPHUS FEVER IN THE BALKANS

Blood Culture	Days Before 1st Colony Appeared	Number of Colonies	Strain
1.	6	11	S1
2.	10	3	S2
3.	No record	No record	S3
4.	..	0	
5.	10	1	S4
6.	..	0	
7.	..	0	
8.	..	0	
9.	..	0	
10.	17	1	S5
11.	..	0	
12.	..	0	
13.	13	1	S6
14.	13	3	S7
15.	..	0	
16.	11	2	S8
17.	..	0	
18.	..	0	
19.	12	1	S9
20.	11	3	S10
21.	10	1	S11
22.	..	0	
23.	..	0	
24.	..	0	
25.	..	0	
26.	9	2	S12
27.	13	3	S13
28.	..	0	
29.	7	2	S14
30.	13	4	S15
31.	..	0	
32.	11	1	S16
33.	13	3	S17
34.	..	0	
35.	19	1	S18
36.	..	0	
37.	..	0	
38.	11	1	S19
39.	..	0	
40.	..	0	

TABLE 3

RESULTS OF BLOOD CULTURES IN TYPHUS FEVER IN RUSSIA

Case	Blood Culture	Day of Disease	Days Before 1st Colony Appeared	Number of Colonies	Amount of Blood Cultured (c.c.)	Strain
1	1	9	14	1	11	R2
2	2	10	10	2	8	R1
3	3	7	..	0	6	
	4	12	9	2	10	R3
4	5	1	6	168	12	R4
	6	5	25 (?)	1	8	R7
	11	11	7	2	13	R9
5	7	8	6	3	10	R5
6	8	4	..	0	15	
	9	6	8	2	11	R6
	15	11	26 (?)	1	10	R10
7	10	6	..	0	9	
8	12	10	..	0	13	
	16	14	..	0	6	
9	13	6	..	0	13	
	18	11	29 (?)	1	12	R12
10	14	6	9	1183	13	R11
11	17	9	7	1	10	R8
12	19	8	14	2	11	R16
13	21	7	6	1	11	R14
14	20	3	..	0	12	
	23	5	7	1	11	R15
15	22	5	..	0	13	
	24	6	9	2	6	R13
	27	11	..	0	9	
16	25	6	24 (?)	1	12	R18
17	26	2	15	1	12	R19
18	28	9	..	0	8	
	32	12	..	0	12	
19	29	5	..	0	10	
20	31	13	6	2	12	R17
21	30	6	..	0	12	
	33	9	..	0	10	
22	34	2	7	1	10	R20
23	35	5	7	2	10	R22
24	36	7	10	8	10	R21

Results of Russian Studies.—Thirty-six blood cultures were made in Volhynia and Galicia on 24 persons suffering from typhus fever. Twenty-two of the 36 blood cultures, or 61%, were positive. In some individuals, however, as many as 3 cultures were made at various times during the disease. In all, therefore, B. typhi-exanthematici was recovered in pure culture from the blood of 19 of the 24 cases of typhus fever, or in 79%.

This percentage is higher than that in the New York typhus, but lower than that in either the Mexican or the severe Balkan 1914-15 type of the disease. For, on comparing the percentage of positive results with the percentage of mortality in the various forms of the disease thus far studied (the Balkan 1915-16 series being omitted for reasons mentioned) the following is noted (Table 4).

TABLE 4

COMPARISON OF PERCENTAGES OF MORTALITY IN VARIOUS FORMS OF TYPHUS FEVER WITH THE PERCENTAGES OF POSITIVE BLOOD CULTURES

Type of Disease	Mortality Percentage	Number of Cases Studied	Percentage with Positive Blood Cultures
New York typhus (Brill's disease)	0.3	34	53
Russian typhus	5.5	24	79
Mexican typhus	20	8	100
Balkan typhus, 1914	18 - 60	7	100

It is apparent from Table 4, that the severer the disease the more readily is B. typhi-exanthematici isolated from the blood. This has also been our experience with the individual cases, the blood cultures in the severer cases being almost uniformly positive, in the milder ones often negative. Among the Russian cases, altho all grades of severity were encountered, many were mild, were in fact, clinically indistinguishable from the type endemic in New York, commonly known as Brill's disease.

In the Russian studies we had the unusual opportunity of taking a culture (No. 5) on a patient on the very first day of the disease. This culture was positive, as were also 2 other cultures (Nos. 26 and 34) made on patients on the second day of illness. It is evident, therefore, that the organism is present in the circulating blood from the onset of the disease.

In this regard it is of interest to note that Culture 31, taken during the last 12 hours of a patient's illness, was also positive. Such results were also reported in the New York studies, and in 2 of the cases of the New York series the organisms could still be recovered from the blood 12 and 36 hours, respectively, after the crisis. All other cultures on convalescents made in New York were negative. In addition to those tabulated in Table 3, blood cultures were also made in the Russian studies on 4 cases during the first week of convalescence, with negative results.

Cultures 5 and 14 being omitted for the present, the total number of colonies developing in the remaining 20 positive cultures in the Russian series was 37. Including therefore both negative as well as positive cultures, we find that 37 colonies developed in 346 c.c. of blood. It seems probable that this may not represent the actual number of bacilli in that amount of blood. As previously stated, it is quite possible that some of the bacilli in the blood do not succeed in adapting themselves to the artificial medium and therefore do not develop into colonies. But comparing these results with those observed in the milder type of the disease (New York typhus) or in the severe forms (Mexico and Balkans, 1914), we note the following (Table 5):

TABLE 5

COMPARISON OF DIFFERENT FORMS OF TYPHUS FEVER IN RESPECT TO NUMBER OF COLONIES IN BLOOD

Type of Disease	Mortality Percentage	Amount of Blood Cultured (c.c.)	Number of Colonies
New York typhus (Brill's disease)........	0.3	436	34
Russian typhus...........................	5.5	346	37
Mexican typhus...........................	20	120	34
Balkan typhus (1914).....................	18 - 60	51	74

From Table 5 it is apparent that there is a definite relationship between the degree of bacteriemia and the severity of the disease.

The typhus seen in the Balkans during 1915-16 was midway in severity between the Russian and the Mexican, the mortality being 11%. Unfortunately, there are no records of the amount of blood used in this series of cultures, so that it could not be included in the table. There were, however, 44 colonies in 18 positive blood cultures, as compared with 37 in 20 positive cultures in the Russian series and 34 in 11 cultures in the Mexican. Apparently, therefore, the Balkan typhus of 1915-16 belongs, in Table 5, between the Russian and Mexican types of the disease, both as regards degree of bacteriemia and severity of the illness.

The results summarized in Tables 4 and 5 demonstrate that the intensity of the bacteriemia runs parallel to the severity of the disease. This observation has previously been made in our American studies.

BLOOD CULTURES DURING CHILLS

In practically every case of typhus fever seen in Russia, a history of an onset with a chill could be elicited. More rarely the disease was ushered in with only chilly feelings. During the course of the disease chills or chilly feelings were more rarely encountered, and as a rule occurred during the first week of the illness.

It was our good fortune to be able to take blood cultures on 2 patients during chills. The first one (Culture 5) was made during the initial chill at the very onset of the illness. The patient, a girl of 9 years, had been admitted to the quarantine ward of the typhus hospital because of the presence of the disease among the other members of her family. At the time of her admission, 3 older sisters and a brother were in the second week of their disease, and the mother was in the first week. The father of the family and this little girl were the only members not affected by the disease at the time of her admission. During the next week both remained apparently in good health and their temperatures, taken 3 times a day, were always normal. On the evening of the 18th of January, 9 days after admission to the hospital, the girl complained of a headache and slight chill. Her temperature at this time was normal. On the morning of the 19th she again felt perfectly well and looked well, but her temperature was 37.5 C. (99.5 F.). The slight chill and headache of the previous evening were suspiciously like the prodromal symptoms of typhus fever, so that it was thought advisable to move the patient to the typhus observation ward. In the evening the temperature rose to 38.5 C. (101.2 F.) and there was again a slight headache. On the morning of the 20th the temperature was down to 38 C. (100.4 F.), and the child again looked and felt well. Toward noon, however, there suddenly occurred a chill lasting about 20 minutes, followed by a rise of temperature to 40.2 C. (104.4 F.). The skin became hot, face flushed, tongue coated, and the patient complained of severe frontal headache. From that time on the disease ran the typical course of a case of typhus fever of moderate severity. As is usual in children, there were no mental symptoms. The exanthem first appeared on the evening of the 4th day of the disease and the temperature dropped rather critically to normal on the 15th day. Both clinically and epidemiologically the case was typically typhus fever.

A blood culture was taken during the initial chill, 12 c.c. of blood being used. One hundred and sixty-eight colonies developed in the blood culture, all of which proved to be pure cultures of B. typhi-exanthematici. This was the largest number of colonies which we had thus far observed in any blood culture, even in the severest forms of the disease. We had never previously had the opportunity of taking a blood culture during a chill. Using the same medium and ascitic fluid previously employed, 2nd and 3rd cultures were made on the patient on the 5th and 11th days of her disease. Tho both were positive, they contained only the usual number of colonies, that is, 1 and 2 colonies, respectively.

The second patient was a man of about 38 years. He was admitted to the hospital on the evening of the 5th day of the disease with a temperature of 39.5 C. (103 F.) and a rather widely spread but fresh exanthem. On the following morning his temperature was 39.2 C. (102.2 F.). In the afternoon of the same day (6th day of the disease) he suddenly had an exceedingly severe chill which lasted almost 2 hours, following which his temperature rose to 40.2 C. (104.4 F.). The chill was so severe that toward the end of it the patient looked as if he were going into a collapse. His face was pinched and slightly cyanosed, and his pulse was thready and rapid. From this time on, the disease ran a very severe course. There were marked delirium and prostration. The temperature began to break on the 10th day of the disease and fell to normal on the 13th day. Convalescence was very much delayed, the patient remaining feeble and emaciated for over a month.

A blood culture (No. 14) was taken during the chill, 13 c.c. of blood being cultured in 6 tubes. On the 9th day thereafter all the tubes were found to be uniformly studded with colonies. Eleven hundred and eighty-three colonies developed, all of characteristic appearance but smaller than usual. Twenty of these colonies were subcultured, and proved to be pure cultures of B. typhi-exanthematici. This blood culture contained the largest number of colonies thus far observed, 91 colonies to the cubic centimeter.

It is of some importance to note that on the same afternoon that this culture (No. 14) was taken, 2 other blood cultures (Nos. 13 and 15) were taken with the same medium and ascitic fluid. Both were from patients who were also suffering from typhus fever. One was made immediately before the blood culture described, the other immeditely after. In Culture 13, 13 c.c. of blood were cultured in 6 tubes; in No. 15, 10 c.c. in 4 tubes. Culture 13 was observed for 26 days and was negative. Blood-Culture 15 was positive, but contained only 1 colony of B. typhi-exanthematici. These two cultures may serve as controls for the media used in Culture 14.

SUMMARY

The bacteriologic studies carried out in the Balkans and Russia on 64 individuals with typhus fever confirmed the observation previously made in New York, that B. typhi-exanthematici is present in the blood during the febrile period of the disease. Up to date, therefore, this organism has been found in pure culture in the blood of typhus-fever patients in the United States, Mexico, Serbia, Bulgaria, Austria, and Russia.

In Serbia and Bulgaria anaerobic cultures were made in 40 cases, and B. typhi-exanthematici isolated from the blood in 19, or 47.5%. Many of the negative cultures had not been incubated properly, and such cultures have undoubtedly materially reduced the actual percentage of positive results.

In Volhynia (Russia) and Galicia positive cultures were obtained in 19 of 24 cases studied bacteriologically, or in 79%.

The organism is present in the blood in typhus fever from the first day of the disease, and can be recovered from the blood during the entire febrile course of the illness.

The intensity of the bacteriemia runs parallel with the severity of the disease.

In 2 instances, blood cultures were made during a chill — in one case at the very onset of the illness — and the blood was found to contain enormous numbers of bacteria.

These observations confirm and strengthen the evidence already presented that Bacillus typhi-exanthematici is the etiologic agent in typhus fever.